Amendments to the Claims

Please cancel claims 28, 38, 39, 58 and 59 without prejudice to subsequent renewal or future prosecution. Please amend the other pending claims as indicated below. The following listing of claims will replace all prior versions, and listings, of claims in the application.

Listing of Claims

1. **(currently amended)** An *Escherichia coli* host cell that comprises:

a) a recombinant solubility reporter nucleic acid construct that comprises a ~~prokaryotic~~ protein solubility responsive promoter isolated from *Escherichia coli* that is operably linked to a reporter gene; and

b) a second ~~target polypeptide-expressing~~ nucleic acid construct that comprises a polynucleotide that encodes a target polypeptide heterologous to the host cell;

wherein expression of the target polypeptide in an insoluble form causes a change in expression of the reporter gene.

2. (previously amended) The host cell of claim 1, wherein the solubility responsive promoter comprises a polynucleotide sequence that is at least 95% identical to a polynucleotide selected from the group consisting of SEQ ID NOS:1-22.

3. (original) The host cell of claim 2, wherein the solubility responsive promoter comprises a polynucleotide selected from the group consisting of SEQ ID NOS:1-22.

4. (original) The host cell of claim 1, wherein the solubility responsive promoter comprises a polynucleotide that comprises a regulatory region of a gene listed in Table 1.

5. (original) The host cell of claim 1, wherein the solubility responsive promoter comprises a polynucleotide that comprises an RpoH recognition site.

6. (previously amended) The host cell of claim 5, wherein the solubility responsive promoter comprises a polynucleotide that is at least 95% identical to a polynucleotide selected from the group consisting of SEQ ID NOS:23-43.

7. (original) The host cell of claim 6, wherein the solubility responsive promoter comprises a polynucleotide selected from the group consisting of SEQ ID NOS:23-43.

8. (original) The host cell of claim 1, wherein the solubility responsive promoter is upregulated when the target polypeptide is expressed in insoluble form.

9. (original) The host cell of claim 1, wherein the solubility responsive promoter is downregulated when the target polypeptide is expressed in insoluble form.

10. (canceled)

11. **(currently-amended)** The host cell of claim 1, wherein the second ~~target polypeptide expressing~~ nucleic acid construct comprises a promoter operably linked to the polynucleotide that encodes the target polypeptide.

12. **(currently-amended)** The host cell of claim 11, wherein the second ~~target polypeptide expressing~~ nucleic acid construct comprises a promoter that is heterologous to the host cell.

13. **(currently-amended)** The host cell of claim 11, wherein the second ~~target polypeptide expressing~~ nucleic acid construct comprises a promoter that is heterologous to the polynucleotide that encodes the target polypeptide.

14. (canceled)

15. (canceled)

16. (canceled)

17. (canceled)

18. (canceled)

19. (canceled)

20. (canceled)

21. (canceled)

22. (original) The host cell of claim 1, wherein the reporter gene comprises a polynucleotide that encodes a selectable or detectable polypeptide.

23. (original) The host cell of claim 22, wherein the selectable or detectable polypeptide is selected from the group consisting: a metabolic enzyme, antibiotic resistance factor, a chemiluminescent protein, and a fluorescent protein.

24. (original) The host cell of claim 23, wherein the detectable polypeptide is β-galactosidase.

25. (original) The host cell of claim 23, wherein the detectable polypeptide is a luminescent or fluorescent protein.

26. (original) The host cell of claim 22, wherein the reporter gene further comprises a polynucleotide that encodes a signal peptide that directs the detectable polypeptide to a surface of the host cell.

27. (original) The host cell of claim 26, wherein the reporter gene further comprises a molecular tag that facilitates separation of a host cell that expresses the reporter gene from a host cell that does not express the reporter gene.

28. **(canceled)**

29. (canceled)

30. (canceled)

31. (canceled)

32. (canceled)

33. (original) The host cell of claim 1, wherein the target polypeptide comprises a mutated form of a polypeptide.

34. (withdrawn) An array of two or more populations of host cells of claim 1, wherein the host cells of each population differ in the target polypeptides expressed by the host cells.

35. (withdrawn) The array of claim 34, wherein the polypeptides differ due to amino acid substitutions, deletions, or insertions compared to a reference amino acid sequence.

36. (withdrawn) The array of claim 34, wherein the target polypeptides expressed by the populations of host cells comprise different fragments of a larger polypeptide.

37. (withdrawn) A method of determining the solubility of a target polypeptide, the method comprising:

a) culturing a host cell of claim 1 under conditions in which the target polypeptide is expressed; and

b) determining whether expression of the reporter gene is increased or decreased, thereby determining the solubility of the expressed target polypeptide.

38. **(canceled)** The method of claim 37, wherein the host cell is a prokaryotic cell.

39. **(canceled)** The method of claim 38, wherein the host cell is an E. coli cell.

40. (withdrawn-previously amended) The method of claim 37, wherein the solubility responsive promoter comprises a polynucleotide sequence that is at least 95% identical to a polynucleotide sequence selected from the group consisting of SEQ ID NOS:1-43.

41. (withdrawn) The method of claim 40, wherein the solubility responsive promoter comprises a polynucleotide sequence selected from the group consisting of SEQ ID NOS:1-43.

42. (canceled)

43. (withdrawn) The method of claim 37, wherein expression of the reporter gene is determined by performing a quantitative assay to determine the amount of detectable or selectable polypeptide in the cell.

44. (withdrawn) The method of claim 37, wherein the host cells are subjected to cell sorting to separate cells having increased or decreased expression of the reporter gene from cells in which expression of the target polypeptide does not change the expression level of the reporter gene.

45. (withdrawn) The method of claim 44, wherein the reporter gene encodes a fluorescent protein and the cell sorting comprises fluorescence activated cell sorting.

46. **(withdrawn-currently amended)** The method of claim 37, wherein: the solubility reporter nucleic acid construct further comprises:

a) a polynucleotide that encodes a molecular tag; and

b) a polynucleotide that encodes a signal peptide;

wherein the signal polypeptide, the molecular tag, and a detectable or selectable polypeptide encoded by the reporter gene are expressed as a fusion protein and the signal polypeptide directs the detectable or selectable polypeptide to a surface of the cell;

and the method further comprises contacting host cells with a solid support to which the molecular tag can bind, wherein cells that express the reporter gene are immobilized on the solid support.

47. (withdrawn) The method of claim 46, wherein the solubility responsive promoter is downregulated when the target polypeptide is expressed in insoluble form, and host cells that express the target polypeptide in insoluble form do not bind to the solid support.

48. (withdrawn) The method of claim 46, wherein the solubility responsive promoter is upregulated when the target polypeptide is expressed in insoluble form, and host cells that express the target polypeptide in insoluble form bind to the solid support.

49. (withdrawn) The method of claim 46, wherein the molecular tag comprises an epitope for an antibody, a poly-histidine tag, or a FLAG™ peptide.

50. (withdrawn) The method of claim 37, wherein the method further comprises:

lysing the host cells under nondenaturing conditions after expressing the target polypeptide, wherein the target polypeptide is in a liquid phase if expressed in soluble form, and in a solid phase if expressed in insoluble form; and

determining the amount of soluble target polypeptide in the liquid phase.

51. (withdrawn) The method of claim 50, wherein the target polypeptide comprises a molecular tag and the method further comprises:

removing an aliquot of the liquid phase after lysing the cells; and

contacting the target polypeptide with a detection reagent that binds to the molecular tag to determine the amount of soluble target polypeptide in the liquid phase.

52. (withdrawn) The method of claim 51, wherein the molecular tag comprises an epitope for an antibody, a poly-histidine tag, or a FLAG™ peptide.

53. (withdrawn) The method of claim 51, wherein the aliquot is placed on a solid support to which the target polypeptide binds prior to contacting the polypeptide with the detection reagent.

54. (withdrawn) The method of claim 53, wherein the solid support is composed of a material selected from the group consisting of glasses, plastics, polymers, metals, metalloids, ceramics, and organics.

55. (withdrawn) The method of claim 54, wherein the solid support comprises a microtiter plate, a nitrocellulose membrane, a nylon membrane, a derivatized nylon membrane, or an agarose particle.

56. **(withdrawn-currently amended)** A method of identifying mutations in a cell that alter the solubility of a target polypeptide comprising:

a) treating a cell with a mutagen;

b) introducing into the cell:

i) a recombinant solubility reporter nucleic acid construct that comprises a protein solubility responsive promoter isolated from *Escherichia coli* that is operably linked to a reporter gene; and

ii) a second ~~target polypeptide expressing~~ nucleic acid construct that comprises a polynucleotide that encodes a target polypeptide;

c) culturing the cell under conditions favorable for expression of the target polypeptide;

d) measuring expression of the reporter gene; and

e) comparing the level of expression of the reporter gene in the cell with the level observed in an unmutated cell that comprises the solubility reporter nucleic acid construct and the second ~~target polypeptide expressing~~ nucleic acid construct to identify a cell that comprises a mutation that alters the solubility of the target polypeptide.

57. **(withdrawn – currently amended)** The method of claim 56, wherein the cell is treated with the mutagen after introducing either or both of the solubility reporter nucleic acid construct and the second ~~target polypeptide expressing~~ nucleic acid construct into the cell.

58. **(canceled)**

59. **(canceled)**

60. (canceled)

61. (withdrawn) The method of claim 56, wherein the solubility is altered to enhance solubility.

62. (withdrawn) The method of claim 56, wherein the solubility is altered to decrease solubility.

63. **(withdrawn-currently amended)** A method for identifying alterations to a polynucleotide that encodes a target polypeptide that alter the solubility of the target polypeptide, the method comprising:

a) altering a polynucleotide that encodes the target polypeptide to form an altered polynucleotide;

b) introducing into a cell:

i) a recombinant solubility reporter nucleic acid construct that comprises a protein solubility responsive promoter isolated from *Escherichia coli* that is operably linked to a reporter gene; and

ii) a second ~~target polypeptide expressing~~ nucleic acid construct that comprises the altered polynucleotide;

c) culturing the cell under conditions favorable for expression of the target polypeptide;

d) measuring the expression of the reporter gene; and

e) comparing the level of expression of the reporter gene with the level observed in a cell with an unaltered polynucleotide that encodes the target polypeptide, to identify an alteration to the polynucleotide that changes the solubility of the encoded target polypeptide.

64. **(withdrawn-currently amended)** A method to identify variations in a ~~process for biosynthesis of a target polypeptide that alter the solubility of the target~~ polypeptide, the method comprising:

culturing a host cell under alternative conditions in which the target polypeptide is expressed, wherein the host cell comprises:

a) a recombinant solubility reporter nucleic acid construct that comprises a protein solubility responsive promoter isolated from *Escherichia coli* that is operably linked to a reporter gene; and

b) a second ~~target polypeptide expressing~~ nucleic acid construct that comprises a polynucleotide that encodes a target polypeptide; and

comparing the expression of the reporter gene by host cells grown under each of the alternative conditions.

65. (withdrawn) The method of claim 64, wherein at least two cells are cultured and the expression of the reporter gene in each cell is compared, thereby identifying a cell that expresses an altered amount of soluble target polypeptide.

66. (withdrawn) The method of claim 64, wherein the protein solubility responsive promoter is upregulated if the target polypeptide is expressed in insoluble form, and expression of the reporter gene at a lower level is indicative of a process condition that results in greater expression of soluble target polypeptide.

67. (withdrawn-previously amended) A method of screening an expression library to identify library members that express soluble target polypeptide, the method comprising:

a) introducing a plurality of expression vectors that each comprise a polynucleotide that encodes a target polypeptide into a plurality of host cells to create an expression library, wherein the host cells comprise a recombinant solubility reporter nucleic acid that comprises a protein solubility responsive promoter isolated from *Escherichia coli* that is operably linked to a reporter gene;

b) culturing the host cells under conditions in which the target polypeptides are expressed; and

c) detecting expression of the reporter gene, thereby identifying library members that express soluble target polypeptides.

68. (withdrawn) The method of claim 67, wherein the protein solubility responsive promoter is upregulated when the target polypeptide is expressed in insoluble form, and host cells that express soluble target polypeptides express the reporter gene at a decreased level compared to host cells that express insoluble target polypeptides.

69. (withdrawn) The method of claim 67, wherein the protein solubility responsive promoter is downregulated when the target polypeptide is expressed in insoluble form, and host cells that express soluble target polypeptides express the reporter gene at an increased level compared to host cells that express insoluble target polypeptides.

70. (withdrawn) The method of claim 69, wherein the reporter gene comprises a selectable marker and host cells are grown under selective conditions, thereby selecting for host cells that express soluble target polypeptides.

71. (withdrawn-previously amended) A method of identifying an antibiotic agent, the method comprising:

contacting a cell that comprises a solubility reporter nucleic acid with a candidate antibiotic agent, wherein the solubility reporter nucleic acid comprises a recombinant protein solubility responsive promoter isolated from *Escherichia coli* that is operably linked to a reporter gene; and

detecting the level of expression of the reporter gene, wherein a change in the expression level of the reporter gene in a cell contacted with the candidate antibiotic agent, compared to reporter gene expression level in a cell which is not contacted with the candidate antibiotic agent, is indicative of an agent that inhibits protein folding in the cell.

72. (withdrawn) The method of claim 71, wherein the protein solubility responsive promoter comprises a polynucleotide that comprises a regulatory region of a gene listed in Table 1.

73. **(withdrawn-currently amended)** A method of identifying a prokaryotic promoter that is differentially regulated in response to expression of an insoluble polypeptide in a host cell that comprises the promoter, the method comprising:

a) providing a host cell that comprises:

i) a solubility reporter nucleic acid construct that comprises a putative prokaryotic protein solubility responsive promoter operably linked to a reporter gene; and

ii) a second ~~target polypeptide-expressing~~ nucleic acid construct that comprises a polynucleotide that encodes a target polypeptide;

b) culturing the host cell under conditions in which the target polypeptide is expressed in insoluble form; and

c) determining whether expression of the reporter gene is increased or decreased, thereby determining whether the putative protein solubility responsive promoter is differentially regulated in response to expression of an insoluble polypeptide in the host cell.

74. (withdrawn) The method of claim 73, wherein the putative protein solubility responsive promoter is a heat shock promoter.

75. (canceled)

76. (canceled)